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Analytical Evaluation of GeneXpert CT/NG, the First Genetic Point-of-Care Assay for Simultaneous Detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis*

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GeneXpert CT/NG was evaluated with 372 characterized bacterial strains. Sensitivity of 10 genome copies/reaction was obtained for both agents. Four *Neisseria mucosa* and two *Neisseria subflava* isolates were positive for one of two gonococcal targets; however, the assay flagged all as negative. The assay was analytically highly sensitive and specific.

Point-of-care (POC) assays which are affordable, sensitive, specific, user friendly, rapid and robust, equipment free, and deliverable (ASSURED) (1) are needed in many settings for the accurate diagnosis of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections. In the past, POC tests have generally relied upon lateral flow technology, and most tests have demonstrated suboptimal performance (2, 3). Cepheid GeneXpert CT/NG is a new Food and Drug Administration (FDA)-approved rapid molecular assay for simultaneous detection of *N. gonorrhoeae* and *C. trachomatis*. This assay is the first genetic point-of-care assay that amplifies one chromosomal target (CT1) for the detection of *C. trachomatis*, two chromosomal targets (NG2 and NG4) for detection of *N. gonorrhoeae*, a single-copy human gene which should be present in each specimen to act as a sample adequacy control (SAC), and *Bacillus globigii* DNA added to each cartridge to serve as a sample-processing/internal control (SPC).

Prior to implementing any assay for diagnostics, it is important to comprehensively evaluate its performance. As Cepheid GeneXpert CT/NG is a molecular assay, consistent with evaluations of laboratory-based nucleic acid amplification tests (NAATs), it should initially be evaluated with characterized strains to ensure that the analytical sensitivity and specificity are optimal. Several NAATs, including commercial assays, have experienced problems with false positivity and false negativity due to cross-reaction (4–7), mutations (8, 9), and genetic exchanges with related organisms (10–12). In particular, diagnosis of extragenital specimens, such as pharyngeal and rectal samples, can result in suboptimal specificity due to cross-reaction with commensal *Neisseria* species or *Neisseria meningitidis* (4, 5, 7, 13–15), which result in low positive predictive values, especially in low-prevalence populations.

In order to ensure accuracy of NAAT results, strategies such as utilization of dual targets in the assays (16, 17) or inclusion of a supplemental "confirmation" have been incorporated in the testing protocol (15, 18, 19). These approaches are, in particular, important for detection from the rectum and oropharynx, which is crucial particularly in population groups of men who have sex with men (20) or with history of oral and/or anal sex. Currently, extragenital specimens are not licensed for use by the FDA; however, several studies have conducted an evaluation and shown appropriate sensitivity with these types of samples using the available commercial NAATs (21–28). Nevertheless, most studies evaluating the sensitivity and specificity of the gonococcal and chlamydial NAATs utilize clinical samples whereby the gold standard for confirmation of *N. gonorrhoeae* is a consensus NAAT gold standard (22). In some cases, cross-reactions are not evident, particularly if the gold-standard NAATs also cross-react with the nongonococcal isolate, giving a false-positive result in the assay.

In this study, we evaluated the analytical sensitivity and specificity of this new POC assay, in particular to evaluate cross-reactivity with nongonococcal *Neisseria* species or other closely related bacteria.

N. gonorrhoeae isolates and isolates of nongonococcal *Neisseria* or closely related bacterial species were obtained from two international *Neisseria* reference laboratories to allow more geographically, temporally, and genetically diverse isolates to be tested. There were 89 isolates from the Neisseria Reference Laboratory at the World Health Organization Collaborating Centre for STD in Sydney, Australia, and 246 isolates from the WHO Collaborating Centre for Gonorrhoea and other STIs, Swedish Reference Laboratory for Pathogenic Neisseria, Örebro, Sweden. Assay performance was evaluated using 111 well-characterized ATCC and clinical isolates of *N. gonorrhoeae* (including 3 mutants with an *N. meningitidis porA* gene) (10, 12) from Asia, Asia-Pacific, Australia, North America, South America, Africa, and Europe. In addition, we tested 236 isolates of nongonococcal *Neisseria* species or closely related bacterial species and 25 *C. trachomatis* strains representing all 15 main serovars (Table 1). All gonococcal and chlamydial isolates were prepared and placed at −80°C prior to testing as described previously (5, 29).

The *N. gonorrhoeae* and *C. trachomatis* genome copy numbers were determined for each DNA extract by quantitative PCR

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TABLE 1 Positivity rate for the detection of characterized isolates of *Neisseria gonorrhoeae*, nongonococcal *Neisseria* and closely related species, and *Chlamydia trachomatis*

Bacterial species tested	No. of isolates tested	No. of GeneXpert CT/NG positive results	
		CT	NG
N. gonorrhoeae	108	0	108
N. gonorrhoeae porA pseudogene mutant[a]	3	0	3
All *N. gonorrhoeae*	111	0	111
N. animalis	1	0	0
N. caviae	1	0	0
N. cinerea	15	0	0
N. elongata	1	0	0
N. flava	1	0	0
N. flavescens	6	0	0
N. kochii[b]	4	0	0
N. lactamica	30	0	0
N. meningitidis[c]	97	0	0
N. mucosa[d]	11	0	0[d]
N. perflava	1	0	0
N. pharyngis	1	0	0
N. polysaccharea	2	0	0
N. sicca	4	0	0
N. subflava[e]	42	0	0[e]
N. weaveri	1	0	0
Other commensal *Neisseria*[f]	5	0	0
Kingella denitrificans	3	0	0
Moraxella catarrhalis	6	0	0
Moraxella osloensis	1	0	0
Other *Moraxella* species[f]	3	0	0
All commensal isolates	236	0	0
C. trachomatis serovar A	1	1	0
C. trachomatis serovar B	1	1	0
C. trachomatis serovar Ba	1	1	0
C. trachomatis serovar C	1	1	0
C. trachomatis serovar D	1	1	0
C. trachomatis serovar E	1	1	0
C. trachomatis serovar F	1	1	0
C. trachomatis serovar G	1	1	0
C. trachomatis serovar H	1	1	0
C. trachomatis serovar I	1	1	0
C. trachomatis serovar J	1	1	0
C. trachomatis serovar K	1	1	0
C. trachomatis serovar L1	2	2	0
C. trachomatis serovar L2	2	2	0
C. trachomatis serovar L2b	1	1	0
C. trachomatis serovar L3	2	2	0
C. trachomatis serovar nvCT 2006-2011[g]	6	6	0
All *C. trachomatis*	25	25	0

[a] Representing three isolates that harbor an *N. meningitidis porA* gene sequence instead of the gonococcal *porA* pseudogene (10, 12).
[b] *N. kochii* (or *N. gonorrhoeae* subspecies *kochii*) is a very rare commensal organism.
[c] Representing isolates of all major meningococcal clones spreading worldwide, including serogroups A, B, C, W-135, Y, and 29E.
[d] Four out of 11 *N. mucosa* strains amplified 1 (NG4) of the 2 NG targets.
[e] Two out of 41 *N. subflava* strains amplified 1 (NG4) of the 2 NG targets.
[f] *Neisseria* or *Moraxella* species that were not able to be identified to the species level.
[g] Representing the Swedish new variant of *C. trachomatis* (8, 9) isolates obtained over a 6-year period.

(qPCR) in conjunction with a quantified standard (Advanced Biotechnologies Inc. [ABI], Columbia, MD). To each cartridge, 1 ml of GeneXpert CT/NG swab transport reagent, 5 μl of extracted DNA from quantitated bacterial strains, and 2 μl of A549 (human alveolar basal epithelial) cell line DNA were added to simulate sample conditions. Approximately 1×10^5 copies of the *N. gonorrhoeae* genome, 2×10^5 copies of other *Neisseria* or related strains, and 5×10^4 copies of *C. trachomatis* were tested in each cartridge.

Strict procedures were followed to avoid specimen contamination and carryover. All strains were deidentified and blind tested.

All 111 *N. gonorrhoeae* isolates were positive for both NG targets (NG2 and NG4) included in the assay and were interpreted as positive. The limit of detection of *N. gonorrhoeae* was 10 genome copies per reaction. Overall, 236 isolates of nongonococcal *Neisseria* species or closely related species were tested. The results of GeneXpert CT/NG were matched with the corresponding organism (Table 1). None of these isolates tested resulted in false-positive results. However, four out of 11 *Neisseria mucosa* isolates and two of 42 *Neisseria subflava* isolates were positive in one (NG4) of two NG targets. Upon retesting, the NG4 target was still positive for all 6 strains. GeneXpert CT/NG assay software interpreted all of these 6 isolates, reactive to one of the two *N. gonorrhoeae* targets, as negative. Accordingly, the NG4 target appears to have some homology to sequences in some of the *N. mucosa* and *N. subflava* strains. As strains of these two species can be present in extragenital samples, the presence of two targets is especially useful when analyzing such samples. The GeneXpert CT/NG assay was recently evaluated for rectal samples and was shown to have high clinical specificity in this sample type (30).

All 15 serovars of *C. trachomatis* were detectable to 10 genome copies per reaction. The GeneXpert CT/NG assay was also able to detect the Swedish new variant of *C. trachomatis* (nvCT) (8, 9) and the L2b strain. The presence of human DNA in all samples was documented by virtue of the sample adequacy control, and valid test results were generated from each analyte. Assay time was 87 min for each sample.

Like commercial and in-house laboratory-based NAAT systems, the GeneXpert CT/NG assay relies on detection of nucleic acid sequences, and thus, any changes in the target may result in loss of detection. There are several examples of such changes in *C. trachomatis* (8, 9) and *N. gonorrhoeae* (10–12). The closed, self-contained, fully integrated and automated GeneXpert CT/NG system was analytically highly sensitive and specific for detection of all types of genetically different *C. trachomatis* and *N. gonorrhoeae* strains. Also, the incorporation of two *N. gonorrhoeae* targets, both of which would need to be positive for reporting a positive result, allows reporting with more confidence.

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